REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

Initially, Applicants express their appreciation to the Examiner and his supervisor for their courtesy and assistance provided to the Applicant's representative during the personal interview held on March 7, 2003.

A Sequence Listing is added to the application papers, in response to the Examiner's suggestion set forth in item 5.c. on page 4 of the Action. SEQ ID NO: 1 is the sequence of NDPK of *Myxococcus xanthus*, which is described in reference 16 mentioned on page 14 of the specification, as well as being discussed in the paragraph bridging pages 7-8 of the specification. The teachings of the references listed on page 14 are fully incorporated by reference into the application, as noted on line 1 of the page. A copy of reference 16 is enclosed for the Examiner's information. Attention is directed to Figure 4. The same sequence was published in 1990 and is available from Genbank under accession AAA25400, a copy of which is enclosed. SEQ ID NO: 2 is the same sequence as SEQ ID NO: 1, except that the Asp at position 112 has been changed to Cys. See page 8, lines 14-16 of the specification.

A computer readable version of the Sequence Listing is also enclosed. The paper and computer readable copies of the Sequence Listing are identical. No new matter is added.

Claims 20, 21 and 31 have been amended as suggested in items 5.a. and 5.b. on pages 3-4 of the Action. Claims 30 and 35 have been revised to identify the sequence of SEQ ID NO: 2. The objection to claim 30 set forth in item 4. and 5.c. is deemed to be overcome by the foregoing amendments to claim 30.

The objection to claim 30 set forth in item 3., and the rejection of the claims under 35 USC 112, second paragraph, as set forth in item 5, are deemed to be overcome in view of the foregoing amendments.

Claims 20, 21 and 31 have also been amended as discussed during the interview, to specify that the label is an extrinsic label. Support is found in the specification at page 4, line 16 to page 5, line 6.

A new title has been suggested. However amendment of the title is deferred, because the restriction requirement is untenable and is again respectfully traversed.

The basis for maintaining the restriction requirement is that "the inventions of Groups I-III as set forth in Paper No. 6, when considered as a whole, do not contribute over the prior art".

Patentability of the claims is irrelevant to restriction. The Examiner is requested to point out where in the MPEP it is taught that patentability has relevance to determination of unity of invention.

As pointed out in Applicant's last response, the claims 20-38 of Groups I-III possess unity of invention, because they all possess the same or corresponding special technical feature, which is the requirement that the NDPK is modified to carry an extrinsic label in both the NDPK's phosphorylated and unphosphorylated forms, which label gives a different detectable signal when the enzyme is phosphorylated from when it is unphosphorylated. See claims 20, 21 and 31. All claims require this special technical feature. Accordingly, all claims possess unity of invention. This conclusion is clearly supported by Example 6 on page AI-58 of Annex B of the MPEP, a copy of which was submitted with Applicant's last response.

Reconsideration of the restriction requirement is accordingly requested.

Claims 20, 24-27 and 29 are rejected under 35 USC 112, first paragraph, on the basis that the claims lack a sufficient written description, for the reasons set forth in item 6. This ground of rejection is respectfully traversed.

There is a strong presumption that an adequate written description of the claimed invention is present when a patent application is filed. See page 2100-156 of the MPEP, top right column, citing *In re Wertheim*. The Examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. See the sentence bridging pages 2100-168 to 2100-169 of the MPEP. In rejecting a claim, the Examiner must express the following findings of fact:

(1) Identify the claim limitation at issue; and

(2) Establish a prima facie case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure as filed.

The rejection does not clearly identify the claim limitation at issue in the rejection.

Applicant has assumed that the claim limitation is "the NDPK is modified to carry a label", in view of the remark that the specification teaches "the structure of only a single representative species of such modified NDPKs".

The inventors were clearly in possession of the invention as claimed at the time the application was filed. The original claims and disclosure fully teach that the inventors considered their invention to be applicable to any NDPK modified to carry any label.

Moreover, a single species may support a genus. See page 2100-164, right column. One skilled in the art would recognize that the disclosed invention would be applicable to any NDPK modified to carry any label, based upon the single representative species described in the specification, for the following reasons:

- NDPK enzymes hade been cloned and sequenced for a large number of organisms, as detailed on page 2, lines 25*ff*. Despite the natural variation between the various enzymes, they all operate by the same basic mechanism with a phosphoenzyme intermediate.
- The phosphorylation event inherently results in a change in the enzyme's 3D structure. The addition of a large charged group to a protein's active site can have little other effect. It is therefore safe to assume that any NDPK will exhibit a conformational change when it is phosphorylated.
- Protein chemistry offers numerous methods for detecting conformational changes. The application itself describes several methods at page 3, line 26*ff*, relying on both intrinsic properties of the enzyme or on the properties of a modified enzyme. For example, the prior art [ref. 18 in the application] had already described methods in which the phosphoenzyme could be tracked by intrinsic tryptophan fluorescence.
- Extrinsic labels offer the most flexible and convenient way of monitoring conformational changes because an intrinsic label such as the inherent fluorescence of tryptophan residues

has low signal strength and, in addition, suffer from interference from other tryptophan residues. An extrinsic label allows excitation and emission wavelengths to be chosen to suit particular circumstances and offers better quantum yields and/or environmental sensitivity.

- The application teaches that extrinsic labels can be attached to cysteine residues, due to their reactive sulphur atoms. The application further teaches that cysteine residues can, if necessary, be introduced by site-directed mutagenesis. Indeed, mutagenesis was required for the *M.xanthus* enzyme used in the examples.
- NDPK sequences are largely conserved *e.g.* a simple BLAST of the *M.xanthus* sequence against GenBank easily fills the default 100 hits with NDPK sequences, with hit number 100 still having an "expected" value of $4x10^{-32}$ (*i.e.* even at hit 100 the match is extremely high). The application teaches that labels should be attached in the vicinity of the enzyme's active site (see page 8, lines 14-16), and a search of "nucleoside diphosphate kinase" against the 3D structural databases gives more than 30 hits. Residues identified in one 3D structure can therefore easily be mapped to other NDPK enzymes by simple sequence alignment.
- In parallel, the application teaches that any necessary mutations should not disrupt enzymatic activity (page 4, lines 18-20). Reference 20 cited in this section teaches a residue where mutation is <u>not</u> tolerated, and so the skilled person is specifically guided away from modifying certain residues and towards modifying others near the active site. It is strange that the examiner has cited reference 20 [Izumiya & Yamamoto] <u>against</u> enablement when the applicant specifically chose to cite the document to guide the skilled person away from its failure. Similarly, the examiner has cited Schneider *et al.*, but this citation teaches a mutation "near the nucleotide binding site" which is specifically said not to affect the protein's enzymatic characteristics, thus contradicting the Examiner's reasoning.

- The paragraph bridging pages 6 to 7 of the office action mentions various problems which might be encountered when labelling a protein near its active site. However, all of these problems are hypothetical and unproven.
- For instance, the examiner argues that the introduction of a cysteine residue near the active site is unpredictable and might abolish its biological activity. However, the very fact that a suitable residue was identified, mutated, and labelled in *M.xanthus* without extensive effort indicates the contrary *i.e.* the invention can be implemented without undue burden. The inventors' experience with the *M.xanthus* enzyme shows that, while the examiner's problems may exist in theory, they did not exist in real-world practice.
- In fact, because the inventors succeeded in a situation where a mutation had to be introduced near the active site ("...proximity to the nucleotide-binding cleft seen in the crystal structure..." – page 8), the skilled person has more expectation that the invention can be implemented with enzymes which do not require the introduction of new residues. The examiner has argued that mutation followed by labelling in the vicinity of the active site is uncertain, but these are precisely the circumstances where the inventors succeeded. If the inventors succeeded in the most difficult situation envisaged by the examiner then success in less demanding situations must *a priori* be expected.
- The examiner has also complained that the invention encompasses the use of any label. However, fluorophores and chromophores *etc.* tend to function regardless of their covalent attachment to proteins. IDCC is a relatively bulky fluorophore (see figure 1) and, given the success with this label, it is wholly reasonable to expect that other labels would function. There is nothing special about IDCC which makes it uniquely suitable for the invention and so it is fair to extrapolate from IDCC to other labels.
- The examiner has presented no empirical evidence of failure in other enzymes or with modification of other residues or with other labels. Furthermore, the examiner's rational theory of failure is disproved by the inventors' success in the most difficult situation which has been envisaged *i.e.* previously-untested mutagenesis in the vicinity of the active site, followed by covalent attachment of a bulky fluorophore.

- The examiner's theoretical objections might fit a situation where the invention involves an unknown enzyme, unknown techniques and hypothetical examples. This invention, however, uses an enzyme which has been sequenced for multiple organisms, which has had several 3D structural determinations, which uses standard mutagenesis techniques, which uses standard labelling techniques, and which has empirically been shown to work as expected.
- The conclusion must therefore be that the skilled person would be able to modify and label other NDPK enzymes in much the same way as described for *M.xanthus* in order to give suitable reagents for use with the invention. The inventor's single species thus offers sufficient guidance for the whole of the claimed genus.

In summary, the Examiner has provided no evidence, and insufficient reasoning, to support a prima facie ground of rejection of claim 20, and particularly claims 24-27 and 29. Accordingly, reconsideration and withdrawal of this rejection is appropriate and solicited.

Claims 20, 24-27 and 29 are rejected under 35 USC 112, first paragraph, as lacking enablement for the reasons set forth in item 7, for any NDPK modified by any method to carry any label. This ground of rejection is respectfully traversed for the reasons stated above.

The skilled person would be able to modify and label other NDPK enzymes in much the same way as described for *M.xanthus,* and using routine experimentation, test the modified enzymes to identify suitable reagents.

Lastly, claims 20, 24, 27 and 29 are rejected under 35 USC 103 as being unpatentable over Schneider et al. in view of Deville-Bonne et al. for the reasons set forth in item 8. This ground of rejection is respectfully traversed.

Schneider *et al.* is a paper which teaches that *Dictyostelium* NDPK's intrinsic fluorescence correlates with its phosphorylation state and that an extra tryptophan introduced at residue Phe-64 "provides a direct spectroscopic probe" of phosphorylation.

Deville-Bonne *et al.* teaches that the fluorescence of Trp-137 in *Dictyostelium* NDPK can be used "as a sensitive probe for monitoring the interaction of the enzyme with its substrates".

The examiner has combined the two references to argue that it would be obvious to use a *Dictyostelium* NDPK with a Phe-64-Trp mutation to measure ADP levels.

In fact, the applicant already acknowledges that intrinsic fluorescence of tryptophan residues had been used to detect phosphoenzyme in the prior art [Schaertl *et al.*, cited on page 4 as ref. 18], so the examiner's statements are not new to the applicant. The examiner's combination of documents ends up with a method which utilises the intrinsic fluorescence of a mutant residue in an NDPK.

The claims as amended clearly define over such a combination, by clarification that the label is an extrinsic label, as discussed during the interview. There is nothing in the cited prior art which suggests adding an extrinsic label to NDPK in order to probe its phosphorylation status. Extrinsic labels offer the most flexible and convenient way of monitoring conformational changes because an intrinsic label such as the inherent fluorescence of tryptophan residues has low signal strength and, in addition, suffer from interference from other tryptophan residues. An extrinsic label allows excitation and emission wavelengths to be chosen to suit particular circumstances and offers better quantum yields and/or environmental sensitivity. Such benefits are neither disclosed nor suggested in the prior art.

Accordingly, the claims are patentably distinct and nonobvious over the cited references.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

In view of the foregoing, it is respectfully submitted that each ground of rejection set forth in the Official Action has been overcome. Accordingly, reconsideration and allowance is respectfully solicited.